

## Effect of Temperatures of up to 45°C on Survival of Variola Virus in Human Material in relation to Laboratory Diagnosis

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Although considerable advances have been made in the control of some of the more serious diseases caused by infective agents in tropical and sub-tropical climates, smallpox remains an important cause of morbidity and mortality in large areas of several continents. The development of high-quality lymph which is stable in dried form at temperatures of 45°C should help to facilitate control of the disease by more active vaccination programmes.

In other areas where several years may pass without the occurrence of a case it is often difficult to maintain public interest and a satisfactory level of immunity. This state of affairs seems likely to become more widespread as time goes on and, at the same time, extended air travel makes it possible for individuals incubating the disease to arrive and become firmly established in a community by the time they become infectious. If they are infected with alastrim virus, or have a low level of post-vaccinal immunity, the disease may pass unrecognized for several weeks.

It seems likely that more widespread use of laboratory diagnostic tests in suspected cases may aid the health authorities in the control and eradication of the disease. For several years, such a service has been provided in Great Britain not only for indigenous cases, but also by the Central Public Health Laboratory for suspected cases on board ships en route for Great Britain, and for similar patients in Aden, Malta and Gibraltar.

In order that the results of tests may be of value the specimens must be transported to the laboratory under suitable conditions. In olden days powdered crusts or matter from pustules from patients with variola, or vaccinia dried on threads or on ivory points, was sent from one doctor to another for vaccination purposes, indicating the resistance of the virus in this form at temperatures prevailing in temperate climates (maximum about 25°C); less information is available, however, on the survival of these viruses in their "natural state" at higher temperatures. Although inactive preparations of virus may be used for the complement fixation test, this test is quantitatively much less sensitive than the egg culture of live virus—a method which is likewise indispensable for distinguishing between the viruses of variola, vaccinia and cowpox.

The accompanying table is compiled from results accumulated over several years as specimens became available.

Smears on glass slides are a convenient method of collecting material for complement fixation and egg culture, as well as microscopic examination from scrapings of maculopapular lesions. They may be helpful in providing additional material when only one or two vesicles are present and difficulty is encountered in collecting fluid or pus in a capillary. How-

**SURVIVAL AT VARIOUS TEMPERATURES OF  
VARIOLA MAJOR AND ALASTRIM VIRUSES IN MATERIAL FROM PATIENTS**

Type of specimen	Variola major				Alastrim			
	20-25°C	30°C	37°C	45°C	20-25°C	30°C	37°C	45°C
Smears on glass from macule, papule base of vesicle or vesicle fluid	84 *	—	—	—	+	ND	ND	ND
Vesicle or pustule fluid	84 *	4.5 **	4	ND	+	1-2	1 ± †	—
Scab or crust	more than 1 year	+	+	12 †	+	+	+	12 ††

\* From Downie & Dumbell <sup>a</sup>

\*\* Figures relate to days unless otherwise stated

† 2 tests positive, 1 test negative

†† Not tested for longer than 12 days

ND = not tested

ever, it can be seen that the virus collected in this way is particularly vulnerable to the effect of temperatures higher than 25°C.

Smears were available only from variola major patients and had been stored for two years or more at 4°C when tested. The smears were exposed in Petri dishes in an incubator and no virus could be recovered after 24 hours' exposure at 30°C. It is impossible to provide absolute controls for such material from patients, but other smears of similar size and thickness from the same patient showed total pock counts of several hundred.

Virus in smears from patients with vaccinia (vaccinia eczematicum) survived for 24 hours at 30° and 37°C, but not for 48 hours at these temperatures.

As would be expected, vesicle or pustule fluid is more resistant to higher temperatures, but isolation of live virus cannot be guaranteed after exposure for more than two or three days if the results with alastrim, which were based on material from two patients in the same outbreak, are taken into account. The difference between results with alastrim and variola major cannot be explained satisfactorily as quantitative control was not possible, although unheated controls of the same batch were always included. The general impression was that there was less virus in an equal volume of alastrim vesicle or pustule fluid than in similar variolous material. Even at 37°C the variola major virus survived four days, whereas some tests were positive and some negative with alastrim after only 24 hours. It is possible that survival of variola virus in this form may occur even at 45°C but material for such a test has not been available.

The long survival of both viruses in end-stage lesions such as crusts or seeds is well recognized, and resistance up to 45°C shows that this material may always be satisfactorily sent by ordinary post or transport.

<sup>a</sup> Downie, A. W. & Dumbell, K. R. (1947) *Lancet*, 1, 550

Often the patient may not be seen nor the disease suspected until these lesions appear; provided several scabs are available the result is definitely diagnostic.

The observed differences between alastrim and variola major vesicle fluid at 30°C and 37°C suggested the possibility of differentiation between these viruses by tests for thermal stability. Unfortunately, experiments with known quantities of virus in suspensions of infected chorioallantoic membrane heated in ampoules in a water bath at temperatures between 50°C and 58°C has not shown any clear difference.

**Conclusions.** Specimens collected from smallpox patients at any stage of the disease may be transported to the laboratory by hand or post over a period of several days if the temperature is not above 25°C.

If the temperature is 30°C or higher, material collected in the maculopapular stage by scraping lesions or putting a needle through them should be chilled in order to ensure survival of virus.

Virus in vesicle and pustule fluid may survive several days at temperatures of up to 37°C but for long journeys or at temperatures above that the specimens must be packed in ice.

Material collected from patients after the pustular stage will withstand temperatures of up to 45°C for several weeks.

The results of tests on a small number of specimens collected from two patients suffering from alastrim in 1952, and from several patients with variola major in different years, provide a guide to the conditions of temperature and storage necessary for survival of the virus until it reaches the laboratory, where survival is readily maintained at 4°C. Further tests on fresh material collected in different countries might give more accurate information for the survival in any locality.

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## Chemotherapy of Brucellosis

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Since 1948 there has been ample opportunity to study the value of antibiotics in the treatment of human brucellosis. So far, antibiotics of wide spectrum—those of tetracycline base—have proved their usefulness when given alone or associated with other drugs. However, the results of treatment have caused great disappointment because recurrences are quite frequent, and much confusion has been added by improper evaluation of new drugs.

Because of these facts we have been trying, first, to develop methods by which the personal factor in the evaluation of therapeutic results can be eliminated or minimized, and, secondly, to determine the maximum usefulness of antibiotics given alone or in combination. In attempting to

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